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Clinical Research Article

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Genetically Determined Higher TSH Is Associated With a Lower Risk of Diabetes Mellitus in Individuals With Low BMI

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Abbreviations: BMI, body mass index; DM, diabetes mellitus; fT3, free 3,5,3′-triiodothyronine; fT4, free thyroxine; GRS, genetic risk score; GWAS, genome-wide association study; HbA_{1c}, glycated hemoglobin A_{1c}; HPT, hypothalamic-pituitary-thyroid; MR, mendelian randomization; OR, odds ratio; SNV, single-nucleotide variation; T1DM, type 1 diabetes mellitus; TSH, thyrotropin.

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Abstract

Context: Thyroid status is hypothesized to be causally related with the risk of diabetes mellitus (DM), but previous results were conflicting possibly because of a complex interaction between thyrotropin (TSH), body mass index (BMI) and DM.

Objective: This work aims to investigate the causal association between thyroid status with DM and glucose homeostasis and to what extent this association is dependent on BMI.

Methods: A mendelian randomization study was conducted of European-ancestry participants from the UK Biobank population. The present study involved 408 895 individuals (mean age 57.4 years [SD 8.0], 45.9% men), of whom 19 773 had DM. Genetic variants for circulatory TSH, free thyroxine (fT4) concentrations and BMI to calculate weighted genetic risk scores. The main outcome measures included self-reported DM-stratified analyses by BMI. Analyses were repeated for nonfasting glucose and glycated hemoglobin A_{1c} (HbA_{1c}) among individuals without DM.

Results: Genetically determined TSH and fT4 levels were not associated with risk of DM in the total UK Biobank population. However, in analyses stratified on genetically determined BMI, genetically determined higher TSH, and not fT4, was associated with a lower risk for DM only in the low BMI group (odds ratio 0.91; 95% CI, 0.85-0.98 in low

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BMI; *P* value for interaction = .06). Similar results were observed for glucose and HbA_{1c} among individuals without DM.

Conclusion: TSH, but not fT4, is a potential causal risk factor for DM in individuals with genetically determined low BMI highlighting potential protective effects of TSH only in low-risk populations.

Key Words: diabetes mellitus, glucose homeostasis, hyperthyroidism, hypothyroidism, mendelian randomization, obesity, thyroid function

Diabetes mellitus (DM) is a major public health challenge, mainly due to the increased prevalence of obesity (1, 2). DM is a heterogeneous disease that is caused by different mechanisms, notably, insulin resistance in muscle, adipose tissue, and liver, and impaired pancreatic insulin secretion (2).

Obesity is a major risk factor of DM development (3). However, another potential risk factor for DM development is thyroid status, assessed by circulating concentrations of thyrotropin (TSH) and free thyroxine (fT4) (4). Thyroid diseases, characterized by circulating TSH and/or fT4 outside the reference range, frequently coincide with DM (5, 6). Moreover, associations between variation in circulating levels of TSH and fT4 and DM have been reported in observational studies, though results have been inconsistent (7-9).

Studies on causal inference regarding thyroid status and DM are complicated by the complex interplay with obesity. While the association between obesity and DM is evident, associations between obesity and thyroid status are not fully understood. Observationally, obesity is associated with higher levels of TSH (and lower levels of fT4), though the direction of causation is still unclear (10, 11). Contrastingly, previous mendelian randomization (MR) studies found an association between higher genetically determined body mass index (BMI) and higher free 3,5,3′-triiodothyronine (fT3) concentrations, but no association with TSH or fT4 concentrations (12). Furthermore, the effects of TSH on fat deposits identified in basic research (ie, increased lipolysis and lower rates of adipogenesis) indicate that higher levels of TSH might be protective against adiposity (13, 14).

Since not all confounding and causal factors are known and/or measured in observational studies, it remains unclear whether and to what extent thyroid status affects the risk of developing DM and how obesity modifies this effect. In a previous study, we used genetic instruments to ascertain causality for associations of circulating levels of TSH and fT4 with DM, but did not find evidence for causality (15). However, our previous study suffered from some limitations including a limited sample size and lack of sufficiently strong genetic instruments, especially for fT4.

Currently, novel genome-wide association studies (GWASs) have been performed that doubled the number of genetic instruments for TSH and more than quadrupled the number of genetic variants for fT4 (16). Combined with the recent availability of a large sample size of individual participant data in the UK Biobank, we can now readdress this research question in a more rigorous manner. Because the reported relationship between thyroid status and obesity is paradoxical, the possible effect modification by BMI should be considered to study the effect of thyroid status on DM. We hypothesize that obesity has such a strong effect on the development of DM, especially type 2 DM, that it could overshadow other, more subtle potentially causal pathways, such as thyroid status. By stratification on genetically determined BMI, differential effects of thyroid status on DM can be assessed. The present study aimed to investigate the association between thyroid status and glucose homeostasis and the risk of DM in UK Biobank participants of European ancestry. In addition, we stratified our analyses based on genetically determined BMI to test our hypothesis of a possible effect modification by obesity on the association between thyroid status and DM and glucose homeostasis.

Materials and Methods

Study Population

For the present study, we included all European-ancestry participants from the UK Biobank with imputed genotype data and self-reported data on DM diagnosis. Between 2006 and 2011, men and women aged 40 to 69 years living within a reasonable traveling distance of one of the 22 assessment centers in the United Kingdom were invited to participate in the UK Biobank via a population-based register (17). During their visits to the assessment centers, participants completed questionnaires using a touchscreen device regarding current health and medical history. BMI was established by dividing the weight in kilograms by the height in meters squared. Participants were asked to remove their shoes and heavy clothing before weighing (18). Medication use was assessed by a trained research nurse; for the present study we reported use of levothyroxine

(thyroid hormone supplementation). The UK Biobank operates within the terms of an ethics and governance framework and all participants provided signed written informed consent (18, 19).

Design

MR uses genetic variants as instrumental variables to investigate associations free from most confounding (20). Thus, the exposure is approximated based on genetic predisposition instead of an observed value. In the present study genetically determined TSH and fT4 are used as determinants, but also genetically determined BMI for stratification and effect modification. This design was described previously as a factorial MR (21). Through this factorial design, the association of one risk factor (eg, TSH) can be investigated in the absence or presence of another risk factor (eg, high BMI). Though the genetically determined categories are not directly translatable to clinical categories, they are appropriate for etiological research such as the present study.

Genotyping and Genetic Imputations

UK Biobank genotyping was conducted by Affymetrix using a bespoke BiLEVE Axium array for approximately 50 000 participants; the remaining participants were genotyped using the Affymetrix UK Biobank Axiom array. Quality control was centrally executed by UK Biobank. More information on the genotyping processes can be found online (https://www.ukbiobank.ac.uk). Based on the genotyped single-nucleotide variations (SNVs; formerly single-nucleotide polymorphisms [SNPs]), UK Biobank resources performed centralized imputations on the autosomal SNVs using the UK10K haplotype (22), 1000 Genomes Phase 3 (23), and Haplotype Reference Consortium (HRC) reference panels (24). Autosomal SNVs were prephased using SHAPEIT3 and imputed using IMPUTE4. In total, approximately 96 million SNVs were imputed. Related individuals were identified by estimating kinship coefficients for all pairs of samples using only markers weakly informative of ancestral background.

Selection of Single-Nucleotide Variations Associated With Thyrotropin, Free Thyroxine, and Body Mass Index

For this study, we selected genetic instruments from published GWASs in which the UK Biobank did not contribute. For thyroid status, we selected the lead SNVs for all genetic loci that have been shown to be independently associated with the circulating levels of TSH (42 loci) or fT4 (21 loci) ($P < 5 \times 10^{-8}$) as genetic instrumental variables for TSH and fT4 levels, respectively (25). To investigate the combined effect of the thyroid hormone–associated risk variants, we calculated a weighted genetic risk score (GRS) for circulating TSH or fT4. For the TSH GRS, we excluded rs13100823 mapped to *IGF2BP2*, because this locus has been associated with type 2 DM (25). For the fT4 GRS, we excluded rs11039355 mapped to *FNBP4* because of its previous association with body height, BMI, and proinsulin (25). In addition, we calculated a weighted GRS for BMI, for which we selected the lead SNVs for 97 BMI-associated loci (26). We excluded the rs7903146 polymorphism mapped to *TCF7L2* given its pleiotropic effect on DM risk. For the present study, we considered a low and high genetically determined BMI that were defined based on the median value in the study population.

Outcome Definition

To define cases with DM, the baseline self-reported interview data collected in the full UK Biobank population was used. All participants reporting to have DM were considered cases. Moreover, individuals were asked about their age of diagnosis and whether they used insulin or insulin-analogues within the first year after diagnosis. Given the acknowledged heterogeneity among DM patients, we performed additional exploratory analyses in which we subdivided the DM cases based on the age of diagnosis (given the assumed changing pathophysiology of DM with increasing age) and insulin dependency (27, 28). Using this strategy, we homogenized the case population. These subdivisions were based on the median age of diagnosis (low/high) and the self-reported use of insulin or insulin analogues within the first year after diagnosis (yes/no). Blood samples were drawn in a nonfasted state at the assessment center and stored at –80 °C. Measurements were later performed in random batches. Glucose was measured by hexokinase analysis on a Beckman Coulter AU5800; valid baseline measurements were available for 429 557 participants. Glycated hemoglobin A_{1c} (HbA_{1c}) was measured by high-performance liquid chromatography analysis on a Bio-Rad VARIANT II Turbo; valid baseline measurements were available for 466 492 participants. To identify occult cases of DM for sensitivity analyses, participants who did not report having DM but had either HbA_{1c} greater than or equal to 48 mmol/mL or glucose greater than 11 mmol/L were also marked as having DM. Participants who did not report having DM and had both HbA_{1c} and glucose below these thresholds were used as controls for these sensitivity analyses.

Statistical Analysis

Characteristics of the study population were expressed as mean with SD for normally distributed measures, and proportions for categorical variables.

We performed multivariable logistic regression analyses to assess the association between the GRSs and DM (subtypes), and linear regression analyses were performed for the associations between the GRSs and the continuous variables glucose and HbA_{1c} adjusted for age, sex, and 4 principal components. The resulting estimate is a weighted mean estimate and reflects a SD increase of genetically determined TSH or fT4 on an odds ratio (OR) or unit (mmol/L for glucose and mmol/mL for HbA_{1c}, respectively) increase of our study outcome. To investigate possible effect modification by BMI, our analyses were additionally stratified based on the median of the GRS for BMI. Effect modification by sex was assessed by performing the main analyses stratified for men and women. To investigate whether the presence of thyroid disease could explain sex-specific association, participants using levothyroxine were omitted in sensitivity analyses. To formally test for interaction of the GRSs with BMI, we added an interaction term between the thyroid GRS and BMI GRS in their association with DM to the regression models.

All statistical analyses were performed using R statistical software version 3.5.3 (29). Results were reported as ORs (for dichotomous outcomes) or β estimates (for glucose and HbA_{1c}) with 95% CI.

Results

Population Characteristics

After excluding individuals lacking genetic information or those who were of non-European ancestry, this study comprised 408 895 participants with a mean age of 57.5 ± 8.0 years, of whom 45.9% were men. A total of 19 773 individuals (4.8%) reported a diagnosis of DM. The population characteristics of the study population are shown in Table 1 for controls and DM cases. As compared to controls, DM cases had a higher mean age at center visit (60.6 ± 6.9 years vs 57.3 ± 8.0), were more frequently men (61.8% vs 45.1%), and had a higher mean BMI (31.5 ± 5.8 vs 27.2 ± 4.6). Levothyroxine use was reported by 27 084 (5.6%) participants, more commonly by those with DM than without (8.7% vs 5.4%) and more by women than men (8.6% vs 1.9%). Among the participants included in the present study, nonfasting glucose and HbA_{1c} measurements were available for 356 598 and 389 773 participants, respectively. DM cases had a higher mean nonfasting glucose (7.6 ± 3.4 vs 5.0 ± 0.8 mmol/L) and a higher mean HbA_{1c} (52.4 ± 13.7 mmol/mL vs 35.1 ± 4.5 mmol/mL).

Genetically Determined Thyrotropin and Free Thyroxine With Diabetes Mellitus

A genetically determined higher TSH was not associated with DM in the overall group (OR = 0.96 per SD increase of TSH; 95% CI, 0.92-1.01) (Fig. 1). In the subgroup with low genetically determined BMI, a higher GRS for TSH was associated with a lower risk for DM (OR = 0.91 per SD increase of TSH; 95% CI, 0.85-0.98) (Fig. 1). We did find suggestive evidence for an interaction between genetically determined TSH and genetically determined BMI on DM (*P* value for interaction = .06). No associations between genetically determined fT4 and DM were observed in the main group (OR = 0.96 per SD increase of fT4; 95% CI, 0.90-1.03) and in the group with low (OR = 0.95 per SD increase of fT4; 95% CI 0.84-1.05) and high genetically determined BMI (OR = 0.99 per SD increase of fT4; 95% CI, 0.90-1.08). Also, no formal interaction was present between genetically determined fT4 and genetically determined BMI on DM (*P* value for interaction = .19). When stratified by sex, higher genetically determined TSH was associated with a lower risk of DM in men with low genetically determined BMI (OR = 0.88 per SD increase of TSH; 95% CI, 0.81-0.97), but not in women (OR = 0.97 per SD increase of TSH; 95% CI, 0.86-1.08) (Table 2). Although the effect estimates were different, no formal interaction between genetically determined TSH and sex was observed (*P* value for interaction = .27). Among women, higher genetically determined fT4 was associated with a lower risk of DM (OR = 0.88 per SD increase of fT4; 95% CI, 0.79-0.98), mainly in the women with low genetically determined BMI (OR = 0.83 per SD increase of fT4; 95% CI, 0.70-0.99). Genetically determined fT4 was not associated with risk of DM in men (OR = 1.02 per SD increase of fT4; 95% CI, 0.93-1.11). Thus, the effect estimates for the associations between genetically determined fT4 and DM

Table 1. Characteristics of the study population

	Controls (n = 389 122)	DM (n = 19 773)
Age at study visit, y	57.3 ± 8.0	60.6 ± 6.9
Age at diagnosis, y	–	50.3 ± 14.7
Sex, No., % male	175 670 (45.1)	12 215 (61.8)
BMI	27.2 ± 4.6	31.5 ± 5.8
Levothyroxine use, No., %	21 176 (5.4)	1730 (8.7)
Nonfasting glucose, mmol/L	5.0 ± 0.8	7.6 ± 3.4
HbA_{1c}, mmol/mL	35.1 ± 4.5	52.4 ± 13.7

Data presented as mean with SD or as stated otherwise.
Abbreviations: BMI, body mass index; DM, diabetes mellitus; HbA_{1c}, glycated hemoglobin A_{1c}.

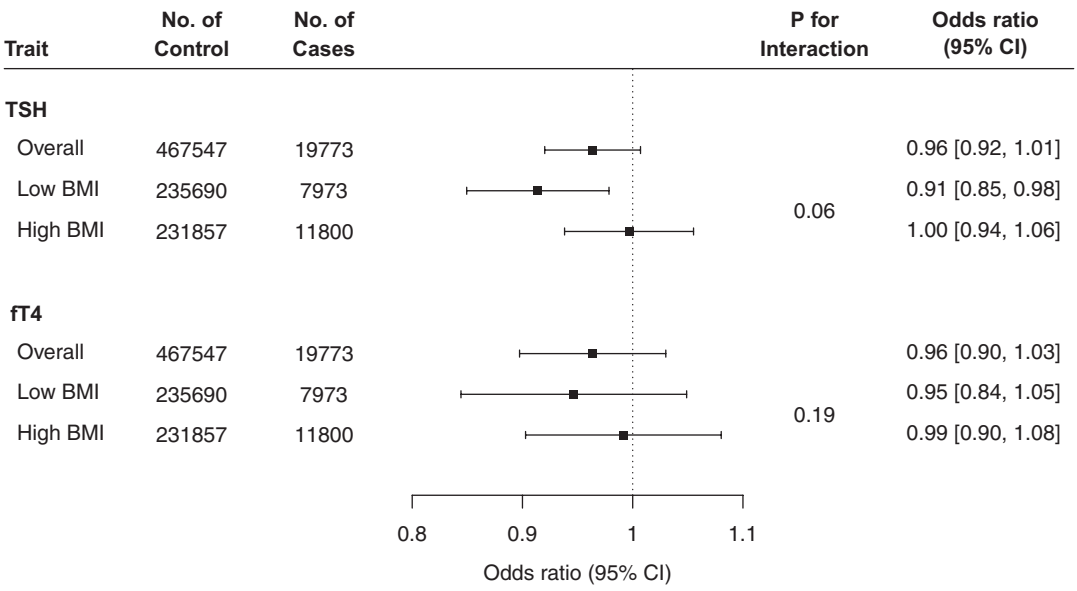

Figure 1. Associations between genetic risk score for thyrotropin (TSH) and free thyroxine (fT4) with diabetes mellitus in the overall population and stratified by genetically determined body mass index (BMI). Odds ratio per genetically determined SD increase of TSH and fT4. Low BMI is defined as genetic risk score (GRS) for BMI below the median, high BMI as GRS for BMI above the median.

Table 2. Associations between genetic risk score for thyrotropin and free thyroxine with diabetes mellitus in men and women and stratified by genetically determined body mass index

	Men				Women				
	No. of controls	No. of cases	OR (95% CI)	*P* for interaction BMI	No. of controls	No. of cases	OR (95% CI)	*P* for interaction BMI	*P* for interaction sex
TSH									
Overall	175 610	12 191	0.95 (0.89-1.00)		213 487	7580	0.99 (0.92-1.06)		.27
Low BMI	88 683	4933	0.88 (0.81-0.97)	.35	108 040	3038	0.97 (0.86-1.08)	.07	
High BMI	86 927	7258	0.99 (0.92-1.07)		105 447	4542	1.00 (0.91-1.10)		
fT4									
Overall	175 610	12 191	1.02 (0.93-1.11)		213 487	7580	0.88 (0.79-0.98)		.05
Low BMI	88 683	4933	1.02 (0.89-1.18)	.42	108 040	3038	0.83 (0.70-0.99)	.24	
High BMI	86 927	7258	1.03 (0.92-1.16)		105 447	4542	0.93 (0.81-1.07)		

OR per genetically determined SD increase of TSH and fT4. Low BMI is defined as genetic risk score (GRS) for BMI below the median, high BMI as GRS for BMI above the median.

Abbreviations: BMI, body mass index; fT4; free thyroxine; OR, odds ratio; TSH, thyrotropin.

differed considerably between men and women, which was reflected by a low *P* value for interaction (.05). Omitting participants using levothyroxine did not materially change the results (data not shown). The results also did not change when 2649 occult DM cases were added to those who reported having DM (data not shown).

Genetically Determined Thyrotropin and Free Thyroxine With Nonfasting Glucose and Glycated Hemoglobin A_{1c}

In individuals without DM, a higher genetically determined TSH was associated with a lower level of nonfasting glucose (β = –0.02 mmol/L per SD increase of TSH; 95% CI,

–0.03 to –0.01) (Table 3). Moreover, a SD higher genetically determined TSH was associated with lower nonfasting glucose levels, in the group of individuals with a low BMI (β = –0.02 mmol/L per SD increase of TSH; 95% CI, –0.03 to –0.01) as well as in the group of individuals with a high BMI (β = –0.02 mmol/L per SD increase of TSH; 95% CI –0.00 to –0.03) (see Table 3). The results for the GRS of TSH on HbA_{1c} showed a similar trend, although with wider CIs. Genetically determined fT4 was not associated with nonfasting glucose or HbA_{1c}.

Exploratory Analyses in Diabetes Mellitus Subtypes

In additional subanalyses of this study, we also explored the associations in DM subgroups based on initiation of treatment with insulin (analogues) within the first year after diagnosis and on the age at diagnosis to assess whether thyroid status was associated with the risk of particular subtypes of DM. Here we did observe an association between a genetically determined higher TSH and a lower risk for DM in patients with a genetically determined low BMI who were diagnosed at a younger age and did not require insulin (analogues) within the first year (OR = 0.87; 95% CI, 0.77-0.98) (see Fig. 2). No other subtypes of DM were identified as associated with either genetically determined TSH or fT4.

Discussion

In this study, we examined the associations between GRSs for circulating TSH and fT4 levels with DM and glucose homeostasis. In the total European-ancestry UK Biobank population, we did not find evidence of an association between genetically determined TSH or fT4 with DM. However, when stratified based on genetically determined BMI, higher genetically determined TSH was associated with a lower risk of DM in the group with a low genetically determined BMI. This association was more pronounced in men than in women. In addition, a higher genetically determined fT4 was associated with a lower risk of DM, regardless of genetically determined BMI, in women but not in men. Along these lines, higher genetically determined TSH was associated with a lower nonfasting glucose level in the overall group and those stratified based on genetically determined BMI.

A strength of this study is the use of a large sample size with a large number of DM cases, which allowed for stratified analyses based on genetically determined BMI and exploratory analyses on DM subgroups. By stratification by genetically determined BMI, the hypothesized catalyzing role of obesity was taken out of the equation, revealing the more subtle causal pathway of the hypothalamic-pituitary-thyroid (HPT) axis on DM. Certain limitations of this study also need to be addressed. The present study made use of self-reported touchscreen-based data, which might be prone to measurement error. Because measurement error is likely to be unrelated to the genetic factors (eg, nondifferential misclassification) this likely resulted in reduced statistical power. Indeed, sensitivity analyses that included participants with suspected DM based on their HbA_{1c} and glucose measurements did not yield different results. Furthermore, because the genetic instrumental variables, as well as the outcome data set, originated from populations of European ancestry, our results may not be generalized to populations of non-European ancestry. In addition, the participants of the UK Biobank are a selected population who are healthier and wealthier than the general population (30), therefore population characteristics are not directly generalizable. Moreover, it was not possible in the present study to account for the heterogeneity of the DM subtypes and type 1 DM (T1DM) because of the lack of a formal diagnosis for T1DM. However, a previous study showed that on average 74% of the diabetes cases diagnosed in those younger than 30 years and 4% older than 31 are likely T1DM cases (31), which would

Table 3. Associations between genetic risk score for thyrotropin and free thyroxine with nonfasting glucose and glycated hemoglobin A_{1c}, stratified by genetically determined body mass index

	Overall		Low BMI		High BMI	
	TSH	fT4	TSH	fT4	TSH	fT4
Nonfasting glucose	–0.02 (–0.03 to –0.01)	–0.00 (–0.01 to 0.01)	–0.02 (–0.03 to –0.01)	0.00 (–0.01 to 0.02)	–0.02 (–0.03 to –0.00)	–0.00 (–0.02 to 0.01)
HbA_{1c}	–0.03 (–0.07 to 0.02)	0.02 (–0.05 to 0.09)	–0.01 (–0.07 to 0.06)	–0.00 (–0.10 to 0.10)	–0.05 (–0.11 to 0.01)	0.05 (–0.04 to 0.15)

Data are presented as SD increase in genetic risk score per SD difference in outcome with accompanying 95% CI. For the analyses on nonfasting glucose, 339 398 individuals are included in the overall analysis: 171 513 in the low BMI group and 167 885 in the high BMI group. A total of 370 859 individuals are included in the overall analysis on HbA_{1c}: 187 503 in the low BMI group and 183 356 in the high BMI group.

Abbreviations: BMI, body mass index; fT4; free thyroxine; HbA_{1c}, glycated hemoglobin A_{1c}; TSH, thyrotropin.

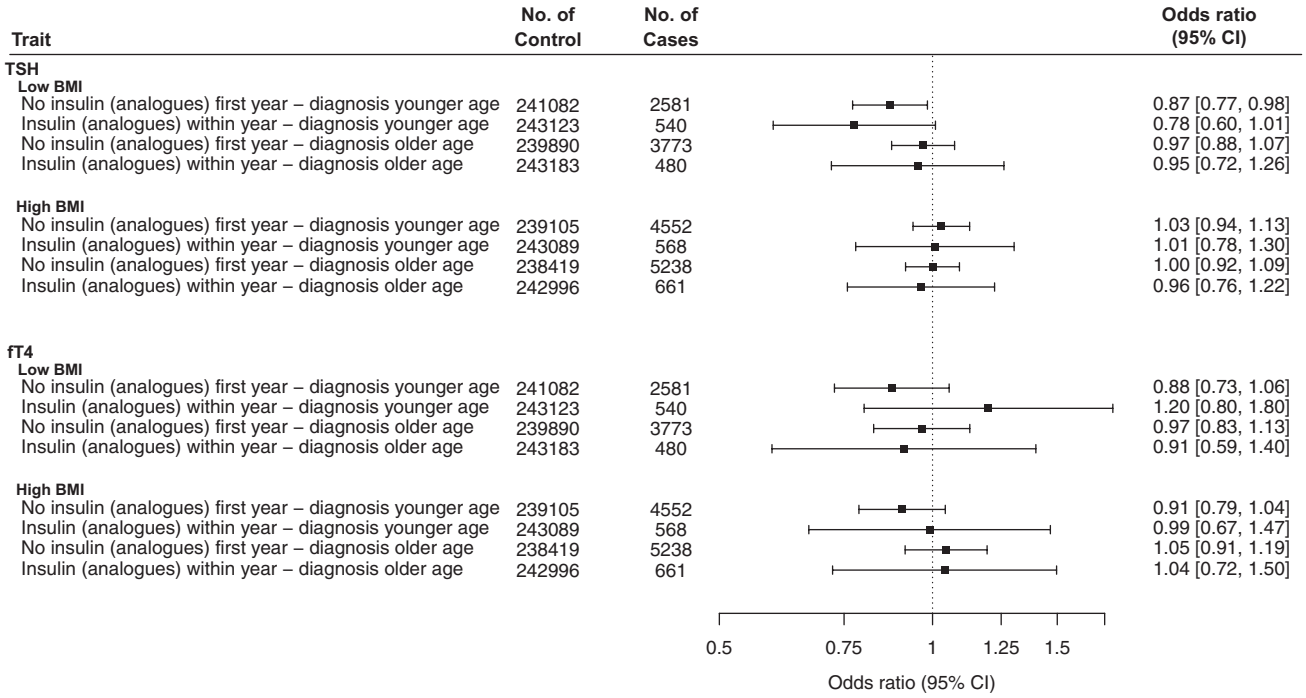

Figure 2. Associations between genetic risk score for thyrotropin (TSH) and free thyroxine (fT4) with subgroups of diabetes mellitus stratified by genetically determined body mass index (BMI). Odds ratio per genetically determined SD increase ofTSH and fT4. Low BMI is defined as genetic risk score (GRS) for BMI below the median, high BMI as GRS for BMI above the median. Diabetes mellitus was specified by whether insulin (analogues) were started within a year after diagnosis of diabetes mellitus and age at diagnosis below or above the median age at diagnosis.

correspond to an estimated total of 2060 (10.4%) cases in our study. Moreover, the use of insulin (analogue) treatment within 1 year after diagnosis also gives an indication for T1DM, which amounted to 2280 (11.5%) individuals. In principle we can state that these are probable T1DM cases and that this heterogeneity in DM cases has been reasonably taken into account by means of stratification based on the use of insulin (analogue) treatments. Mediation by autoimmunity could not be formally assessed because no autoantibodies were available for this cohort. Another limitation of this study is that the role of fT3 could not be investigated. Since fT3 is the active thyroid hormone, it would add to the availability of thyroid hormone in the target tissues; we are thus less able to say anything about this availability in this study.

The findings of the present study add to previous research regarding the role of low thyroid status and DM onset. Several observational studies in humans observed an association of higher TSH level with a higher risk of DM (6, 7). However, not all observational studies showed an association between higher TSH and DM. De Vries and colleagues and Ittermann et al did not observe a relation between plasma TSH levels within the normal range and incident DM (8, 9). The lack of a causal association with TSH and fT4 as observed in the overall study population of the present study may suggest that previously observed associations of alterations in thyroid status and DM onset might have resulted from reverse causality and/or residual confounding. One of the potential interfering mechanisms could be reduced central sensitivity to thyroid hormones commonly seen coinciding with metabolic syndrome (32). In addition, many other factors such as autoimmune disorders could cause residual confounding. Furthermore, these findings confirm our previous observations of no association between circulating TSH and fT4 and risk of DM at population level using 2-sample MR analyses with fewer instruments in a smaller study population (15).

The main novel observation of the present study is the association of higher genetically determined circulating TSH levels with a lower risk of DM in individuals with a lower genetically determined BMI. Since we did not observe any association in those with a high BMI, we hypothesize that the presence of high BMI is such a strong risk factor for DM that more subtle factors of influence are overshadowed. Two main routes of action can be hypothesized: either a direct effect of TSH or an indirect route via a lower HPT axis set point. For TSH to have a direct effect on tissue function, activation of TSH receptors in target tissues is required. Extrathyroidal expression of TSH receptors has been observed in various tissues and cell types, including in orbital fibroblasts, adipose tissue, bone, skeletal muscle, thymus, and kidney (33, 34). TSH could exert

its protective effect against DM via adipose tissue. In mice and human adipocytes, the expression of TSH receptors was demonstrated previously (13, 35). Adipocytes were also shown to increase lipolysis in response to stimulation with TSH in vitro and in vivo (13, 35). Furthermore, interaction with the insulin signaling pathway was demonstrated, leading to an inhibition of phosphoinositide 3-kinase resulting in lower rates of adipogenesis (14). Thus, higher levels of TSH could potentially be protective against accumulation of adipose tissue and thereby reduce the risk of DM. Alternatively, TSH could influence glucose homeostasis through increasing insulin sensitivity and glucose uptake of skeletal muscle. Along these lines, we describe a causal association between higher TSH levels and lower nonfasting glucose levels in this study. Moon et al have demonstrated a direct stimulatory effect of TSH on insulin receptor substrate-1 expression in muscle tissue and improved glucose tolerance (36). Another potential etiological pathway could be via immunomodulation. TSH receptors were shown to be present in thymus tissue, and stimulation with TSH increased development and differentiation of T cells both in rodent and human thymal cell lines (37). Hence, individuals with higher circulating levels of TSH might have a more diverse and effective adaptive immune system. Having a diverse arsenal of T cells prevents autoimmunity and other sources of low-grade inflammation (38). As low-grade inflammation is a well-established causal risk factor for developing DM, any factor targeting inflammatory pathways could be a potential strategy for prevention of DM (39). Higher TSH levels could be such an immunomodulating factor protecting against DM. Apart from direct effects of TSH, an indirect effect of higher TSH via a different HPT axis set point could also explain our findings. As expected from the strong inverse relationship between TSH and fT4, virtually all genetic variants for higher TSH are associated with lower circulating levels of fT4 in the original GWAS (25). Therefore, our observation could be elaborated to an association of higher TSH and lower fT4, that is, a lower HPT axis set point, with a lower risk of DM. Previously, thyroid status has been linked to multiple components of glucose homeostasis. It has long been known that thyroid hormones induce hepatic gluconeogenesis (40). Furthermore, thyroid hormones could affect insulin production and secretion in the pancreas (41). Although thyroid hormones are required for the maturation of pancreatic β cells, senescence is also accelerated by elevated levels of thyroid hormones in these insulin-producing cells (42). Counterintuitively, we found an association only with genetically determined TSH and not fT4. Owing to the smaller number of genetic instruments for fT4 than TSH (20 loci [4.8% explained variance] vs 41 loci [9.4% explained variance]), the statistical power was not as strong for the analyses on fT4. Furthermore, although the genetic instruments for fT4 were strongly associated with circulating fT4 levels, their specific effects on intracellular thyroid signaling in different target tissues are likely heterogeneous and largely unknown.

There was some evidence for sex-specific associations. More specifically, the association of higher TSH and lower risk of DM in people with a low BMI was more pronounced in men than in women. In women, we found an association between higher genetically determined fT4 with a lower risk of DM in the whole group and a slightly stronger effect in the group with lower BMI than higher BMI. In men, this association with fT4 is completely absent. Since medication use for thyroid disease did not explain the sex difference, we hypothesize that the observed sex-specific associations are not due to differences in thyroid disease prevalence but rather differences in body composition between men and women that are not explained by BMI. Men generally have more visceral fat than women, which increases the risk of DM and other cardiometabolic diseases more than overall body fat (43). A potential mechanism for the protective mechanism of TSH is via increasing lipolysis, which might prevent the accumulation of harmful visceral fat in a more relevant magnitude in men than in women. The protective effect of higher fT4 in women and especially in those with low BMI might be related to their low muscle mass. Skeletal muscle is the largest glucose disposal tissue in the body because it aids in the storage and plays a key role in the consumption of glucose; therefore, low muscle mass may impair glucose homeostasis (44). Thyroid hormones induce glucose uptake by skeletal muscles and glucose consumption (45). We hypothesize that thyroid hormone effects on glucose homeostasis of skeletal muscle are noticeable only in those with relatively low muscle mass because the total capacity of larger muscle mass might already suffice. Nevertheless, all these hypotheses are mere speculation and more research is required into sex-specific risk factors and disease mechanisms.

Here, we specifically studied the effects of circulatory TSH and fT4 on DM onset and glucose homeostasis. However, target tissues customize intracellular thyroid hormone levels to their current needs independently of circulating levels in the blood (46). Deiodinases are key players in the modulation of the availability of thyroid hormones in target tissues (47). In previous research by our group, we demonstrated that genetic variation in *DIO1* may affect glucose metabolism (15). This may be more reflective of target tissue levels of thyroid hormone than the circulating levels. We therefore propose that future studies focus on the role of deiodinases and availability of thyroid hormones in target tissues on glucose homeostasis and the risk of DM.

In summary, genetically determined circulating TSH was associated with a lower risk of DM in participants with low genetically determined BMI. In line with this, a higher genetically determined TSH is associated with lower nonfasting glucose levels in participants without DM, regardless of their genetically determined BMI. Although the association with HbA_{1c} was not statistically significant, the results were directionally consistent with the effect estimates for glucose. As they both represent parameters of glucose homeostasis and were moderately correlated ($r = 0.40$), we treated them as complementary measures. We did not find evidence for a causal association between higher circulatory fT4 concentrations and any of our study outcomes. These findings may indicate that TSH levels affect glucose homeostasis and that higher TSH levels might protect against DM. Finding these associations only in subgroups with lower BMI highlights a potential protective effect of TSH only in low-risk populations.

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